SR-FTIR Study of Bacteria-Water Interactions: Acid-base Titration and Silification Experiments

Nathan Yee[1], Liane G. Benning[1], Mark J. Tobin[2], and Kurt O. Konhauser[1]

[1]School of Earth Sciences, University of Leeds, United Kingdom
[2]Synchrotron Radiation Source, Daresbury Laboratory, United Kingdom

Bacterial surfaces are highly reactive and can strongly affect mass transport in a wide range of geological environments. Bacterial cell walls can adsorb aqueous metal cations, and can act as nucleation surfaces for heterogeneous mineral precipitation. However, the reactions at the bacteria-water interface are poorly understood, primarily due to the difficulty in monitoring such processes *in situ* and *in vivo*. In this study, we use synchrotron radiation-based FTIR to investigate the chemistry of bacterial surfaces with acid/base titration and Si precipitation experiments. The objectives of this research are to identify the reactive surface functional groups and to determine how metal adsorption/precipitation affects the protein and lipid structures of individual living bacterial cells. *In-situ* FTIR experiments were performed on the Infrared beamline 1.4.3 at the Advance Light Source (Lawrence Berkeley National Laboratory), using a Nicolet 760 FTIR bench and a Spectra-Tech Nic-Plan IR microscope. All experiments were performed with flow through fluid cell with BaF_2 and ZnSe windows separated by a 6 um mylar spacer. Acid-base titration and Si precipitation experiments were conducted with both intact cells and isolated bacterial sheaths of *Calothrix* (strain KC97) a filamentous cyanobacteria. Titration experiments with intact bacterial cells show a change in peak position of the carboxylic functional group at ~1400 cm^{-1} (symmetric vibrational stretching of deprotonated carboxylate groups) from acidic to near-neutral pH (Fig 1). The bacterial silicification experiments indicate a change in peak position at ~ 1700 - 1740 cm^{-1}, corresponding to the vibrational C=O stretching of esters groups in the lipid structures of the cell (Fig 2). Previous studies have demonstrated that hydrogen bonding onto carbonyl functional

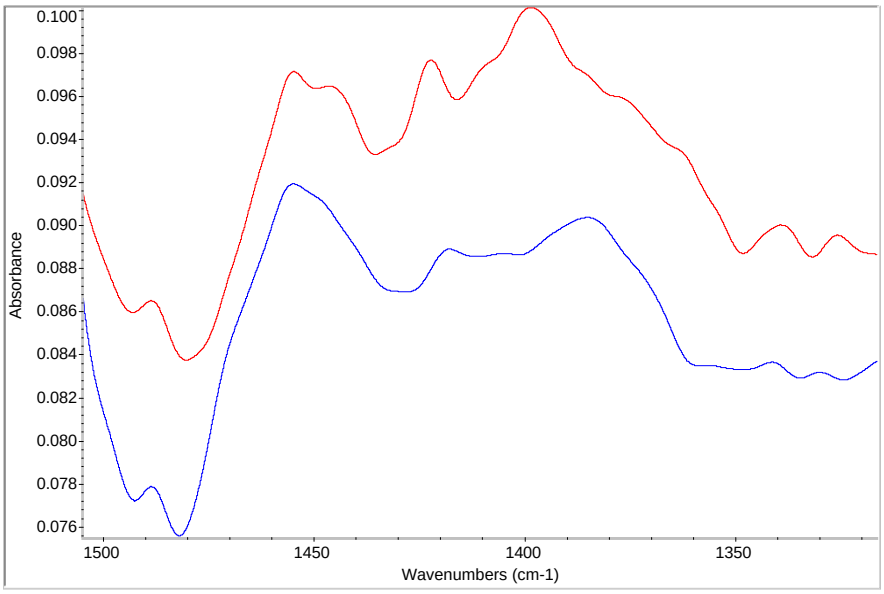

Figure 1. Infrared spectra of an intact *Calothrix* cell in aqueous solution at pH 2.9 and 6.3. A shift in peak position is observed at 1400 cm^{-1} corresponding to $\nu_s(COO^-)$ stretching of deprotonated carboxylate functional groups.

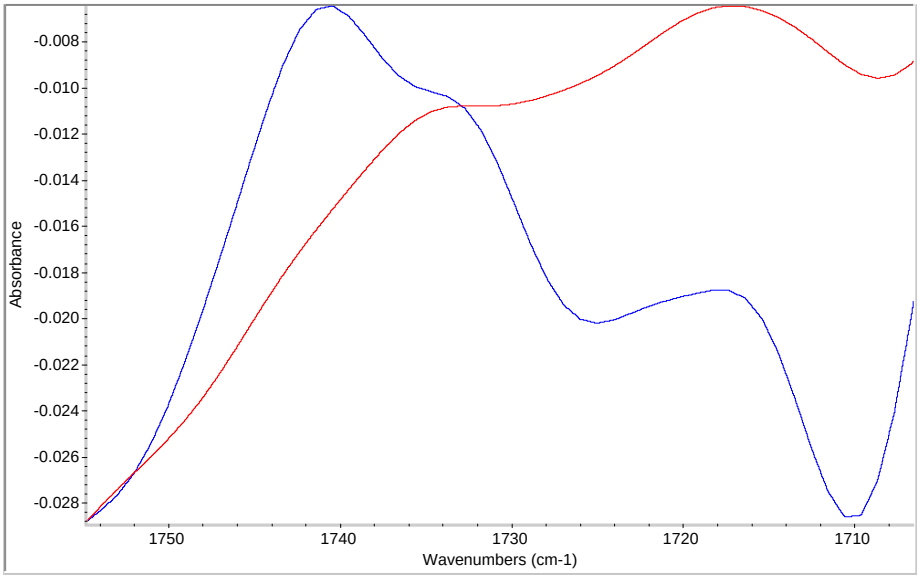

Figure 2. Infrared spectra of an intact *Calothrix* cell before and after silicification. A change in peak position of vibrational C=O stretching is observed.

groups can shift the peak positions in this wavenumber range. Finally, infrared microspectrometry experiments were performed to image the proteins, lipids, and nucleic acids inside intact living cells. Spatial resolution of a few microns was achieved and the chemical distribution of proteins was mapped throughout a *Calothrix* filament (Fig 3). The data indicate that protein molecules have a high concentration within the cell, but a very low concentration on the bacterial surface. These results demonstrate that SR-FTIR can be applied to investigate the functional group chemistry of bacteria in a range of different bacteria-water systems.

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Principal investigator: Liane Benning, University of Leeds, +44-113-233-5220, liane@earth.leeds.ac.uk.

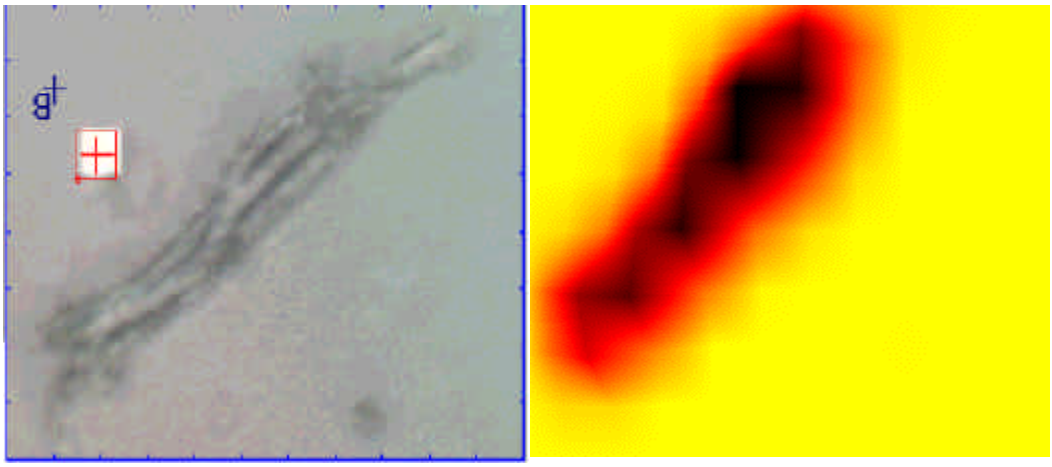

Figure 3. Chemical distribution of the protein characteristic bands amide I and amide II (1495-1800cm^{-1}) of a *Calothrix* filament. a) Optical image, b) 2-D map of the protein distribution.